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Autoradiographic Investigation of the Synthesis
of Ribonucleic Acid in Regenerating Mouse Liver

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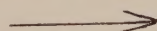
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Abstract

The present investigation studied the synthesis of RNA (and DNA) and the activity of nuclear and cytoplasmic RNase in regenerating mouse liver.

The autoradiographic method was used to study the incorporation of ^3H -uridine into total cell nuclear and cytoplasmic RNA of liver parenchymal cells after partial hepatectomy. After a decreased uptake of uridine into nuclear and cytoplasmic RNA during the first hours, incorporation increased to a maximum and then declined. After RNase-digestion of the liver sections a small incorporation was left, both in nucleus and cytoplasm: RNase-resistant RNA, DNA, or compounds other than nucleic acids. There was a small amount of incorporation of ^3H -thymidine into DNA immediately after partial hepatectomy, but a marked uptake of this precursor was seen 24h after the operation. This time is accepted as the beginning of the S-phase in regenerating mouse liver. The faint labelling of thymidine before the S-phase was correlated with the incorporation of RNase-resistant uridine at the same time. This incorporation could probably reside in metabolic or mitochondrial DNA. The decreased incorporation of uridine into RNA that was found during early regeneration was correlated to an enhanced activity of nuclear RNase at this time. There was no change in cytoplasmic RNase-activity in regenerating mouse liver.


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Autoradiographic and biochemical methods were used to study the incorporation of ^3H -labelled uridine, cytidine, and orotic acid into liver RNA after partial hepatectomy, measured per unit amount of tissue. Nuclear and cytoplasmic RNA labelling was decreased in regenerating liver after injection of uridine. Nuclear RNA labelling was about the same in normal and regenerating liver after injection of cytidine. However, cytoplasmic RNA labelling, which was c. 1 per cent of the nuclear, was twice as high in regenerating as in normal liver after injection of cytidine. No differences between normal and regenerating liver was found on the incorporation into RNA after injection of orotic acid. These autoradiographic results support the biochemical results that none of the precursors has an increased incorporation into cellular RNA 24h after partial hepatectomy.

As rat liver shows an early increase in the incorporation of labelled precursors into RNA, measured per unit amount of tissue, it was suggested that the great demands imposed by regeneration on the increased amount of RNA in mouse liver does not necessitate very large changes in total RNA production. Instead, change in stability leading to less pronounced turn over of RNA is perhaps sufficient.

AUTORADIOGRAPHIC INVESTIGATION OF THE SYNTHESIS OF
RIBONUCLEIC ACID IN REGENERATING MOUSE LIVER

av

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This thesis is based on the following papers referred to in the text by their Roman numerals.

- I. Christina Lorup: An autoradiographic study of the ^3H -uridine and ^3H -thymidine incorporation in the regenerating mouse liver. *Cell Tissue Kinet.* (1977) 10, 477-485.
- II. Christina Lorup: Nuclear and cytoplasmic RNase-activity in regenerating mouse liver. *Virchows Arch. B Cell Path.* (1977) 24, 41-51.
- III. Christina Lorup and Claes Engelbrecht: Incorporation of ^3H -labelled uridine, cytidine, and orotic acid into ribonucleic acid in normal and in regenerating mouse liver - an autoradiographic and biochemical study. In press (*Hoppe-Seyler's Zeitschrift Physiol. Chem.*).

General introduction

In most organs, various functions are carried out by morphologically different cell types; but in the liver, a wide range of functions are performed by one cell type. This varied functional ability might reflect retention of a primitive state of differentiation which enables the hepatic cell to proliferate rapidly after partial hepatectomy or similar stimuli. More information of the control mechanisms responsible for this controlled growth would be of value in understanding the normal regulation of differentiation, proliferation, and growth; also the abnormal growth of neoplasia.

For almost a century (von Podwyssozki, 1886; Ponfick, 1895); in particular since the classical quantitative study by Higgins and Anderson in 1931, it has been known that within a short time the liver can be induced to pass from a "resting" state to one of rapid proliferation that occurs diffusely throughout the residual lobes. Regeneration is the term commonly used to describe the compensatory hypertrophy and hyperplasia that follow hepatectomy. An increased mitotic rate in the hepatic remnant can be observed within 24 hours after operation in the rat (Bucher, 1967), and reconstitution of many hepatic components to their initial (pre-hepatectomy) values can be completed within a few days.

The events leading to such compensatory growth are not fully understood. However, it has been repeatedly demonstrated in cross-circulation (Fisher et al., 1971; Moolten and Bucher, 1967; Sakai, 1970; Short et al., 1972) and in vitro experiments (Demetriou and Levenson, 1977) that there are factors in the blood of partially hepatectomized rats capable of inducing DNA replication in the intact livers of normal animals. Many other factors - such as excess blood

pressure in the remaining hepatic lobes after resection, overloading of the liver remnant with metabolic waste, loss of a growth inhibitor present in the intact liver, and a portal blood hepatotrophic factor - have also been suggested to influence the hepatic regeneration. Insulin and glucagon have been held to be two of the main hepatotrophic factors (Duguay and Orloff, 1977; Lee et al., 1976), together with the more recently discovered epidermal growth factor, which is now also thought to play a part in the normal and regenerating liver (Bucher et al., 1978; Leffert et al., 1979).

Sakai et al., 1977 proposed that there is no single trigger mechanism responsible for the regenerative process. Instead, several factors - such as hepatic inhibitor, splenic inhibitor, intestine associated stimulator, and a pancreatic factor - working together bring about the final outcome. Despite increasingly intense study over the past 15 years, however, it has proved difficult, if not impossible, to obtain quantitative information about the precise rates of synthesis of various hepatic components during regeneration, or even information about the relative roles of changes in rates of synthesis and degradation in accounting for their enormous net increase in mass.

During the early period after partial hepatectomy, there is an activation of the genes; the chromatin template efficiency increases (Bannai and Terayama, 1979; Hwang et al., 1974; Kostraba and Wang, 1973; Thaler and Vilee, 1967) as also does the DNA-template activity of isolated liver nuclei (Pogo et al., 1966). A rapid decrease in chromatin thermal stability occurs in the regenerating liver (Alvarez, 1974; Kusch et al., 1975). During the early period of gene activation in the regenerating liver, there is an increase in the

synthesis of non-histone proteins long before synthesis of histones is initiated (Pogo et al., 1968). This increase in synthesis of non-histone proteins is characterized by the presence of new acidic proteins not found in normal liver (Baserga and Stein, 1971; Dastugue et al., 1971). The positive role played by the non-histone proteins in gene activation (Kostraba and Wang, 1972) suggests that these proteins are involved in the derepressed state of the chromatin during liver regeneration (Tsanev and Sendov, 1971). This pattern of events in the regenerating liver suggests that an extensive "reprogramming" of chromatin structure-function relations occurs as a prelude to DNA-synthesis and cell division.

While in most instances regeneration is known to be accompanied by an increased rate of radioactive precursor incorporation into RNA, the actual magnitude of the observed increase in incorporation often depends on the particular precursor used (Muramatsu and Busch, 1965). Possible accompanying changes in both intracellular and extracellular precursor pool size demand great caution to be used in interpreting results of quantitative studies on the synthetic activity by precursor incorporation. It is of importance that the cellular metabolism and the transport reactions of the precursors during the pulse period are known if the precursors are to provide information about the synthetic activity during regeneration, (Georgiev et al., 1972; Lea and Koch, 1976; Rozet et al., 1974).

One of the first responses to partial hepatectomy is an intensification in the transcribing function of the chromatin. There is an enhanced synthesis of ribonucleic acids, RNAs, in regenerating rat liver (Bucher and Malt, 1971; Bresnick, 1971), and this is associated with an increased activity of RNA polymerase (Kusch et al., 1975;

Organtini et al., 1975). There is also a production of rapidly labelled high-molecular weight RNA in regenerating rat liver nuclei of sequences not produced by normal liver (Mayfield and Bonner, 1972). Molecular hybridization techniques also showed that new species of nuclear RNAs not present in sham-operated controls appeared in the first hours after partial hepatectomy in the mouse (Church and McCarthy, 1967a) and rat liver (Komar and Sedova, 1973; Kostraba and Wang, 1973), thus indicating changes in gene function. Moreover, some RNA molecules present only in the nucleus in normal liver appear in the cytoplasm in regenerating mouse (Church and McCarthy, 1967b) and rat liver (Greene and Fausto, 1977; Schumm and Webb, 1972) and hepatomas (Drews et al., 1968; Shearer and Smuckler, 1971), indicating that the selectivity of RNA transport is impaired in regenerating liver and hepatoma cells. A comparison of RNA isolated at various times during the regenerative process in the mouse showed that the synthesis of different RNA molecules is discontinued at various stages (Church and McCarthy, 1967a). Liver regeneration thus appears to be mediated by short-lived RNA molecules, whose synthesis commences immediately after partial hepatectomy and ceases at various times during the regenerative process. The new species of RNA, which appears before regeneration, can be compared with RNAs associated with rapid cell proliferation in embryonic liver (Church and McCarthy, 1967c). Much of the characteristic regenerating liver RNA thus appears to be the result of reactivation of genes active during liver development but repressed in adult liver.

Most studies of liver regeneration have been made on rats, at which the mentioned phenomena have been observed. This investigation, however, chose the mouse as the experimental animal. As the mouse

is a smaller animal it is likely not to respond exactly in the same way as the rat after liver resection; therefore it would be of interest to study the physiological response of liver resection in the mouse. It is known that the characteristic events of the regenerative process, DNA synthesis and mitosis, start later after partial hepatectomy in the mouse (Lewan, 1972) than in the rat (Grisham, 1962), which possibly would be attributed to differences in the energy metabolism between the animals (Yngner, 1979).

As synthesis and turn over of RNAs seem to be of great importance for the regenerative process in the liver, the present investigation studied the synthesis of RNA in regenerating mouse liver with the aid of radioactive precursors. Nuclear and cytoplasmic labelling of ^3H -uridine into total cell RNA was examined with autoradiography at different times after partial hepatectomy of the mouse (I). For comparison, nuclear labelling of ^3H -thymidine into DNA was also analysed. The incorporation of ^3H -labelled cytidine, uridine, and orotic acid into nuclear and cytoplasmic RNA was analysed with autoradiographic and biochemical methods (III). Finally, the nuclear and cytoplasmic activity of ribonuclease, RNase, was studied (II), it being reported that in some cases degradation of RNA appears to be the stimulus for a regenerative process (Tsanev, 1963). Thus, the RNase-system could possibly be an important controlling factor in growth and cell division.

Material and Methods

Material:

Male or female Swiss albino mice were used. Partial hepatectomy was performed by the method of Higgins and Anderson (1931) under Nembutal or light ether narcosis. The anaesthetic agent was changed during the investigation because ether anaesthesia was of shorter duration than Nembutal, but it was more difficult to regulate at a constant level. Female animals proved more sensitive for the anaesthetic agent than males; therefore female mice were changed for male during the study. The hepatectomies were performed in the morning in order to minimize diurnal effects.

The animals were given ^3H -labelled uridine, cytidine, orotic acid, or thymidine 20, 120, or 300 m. before sacrifice by decapitation at various times after the operation. After sacrifice, the livers were quickly dissected out and prepared for autoradiography, or frozen in liquid nitrogen until the biochemical analyses.

Methods:

RNase-activity (II) was studied spectrophotometrically by measuring the amount of nucleotides obtained after degradation of commercially administered RNA to the liver homogenate by liver RNase. RNA was estimated by UV absorption (II, III). DNA was estimated by diphenylamine reaction (II). Papers (I) and (III) describe the special technique used for autoradiography. Synthesized RNA, registered as silver grains, was measured either per cell (I) or per unit area (III).

Consideration on the autoradiographic method

For detailed information about the autoradiographic technique, see Rogers (1973).

The technique of autoradiography provides a method of detecting radioactivity that differs in several important respects from pulse counting. Compared with the speed and simplicity of the pulse counting method, it is a slow and difficult process to measure the radioactivity with the autoradiographic method. This method is ideally suited to studies of the spatial distribution of radioactivity within a sample, a function that the pulse counters cannot perform. However, the efficiency is lower when measuring the radioactivity of a sample autoradiographically compared with pulse counting.

Irrespective of biological differences in the uptake of tritiated compounds by different types of cells, the radioactivity measured by autoradiography is influenced by a number of factors related to the autoradiographic procedure such as the emulsion, radioisotope, and method used, the speed (sensitivity) of the emulsion, the time of exposure and latent image fading, the resolution, the quantitative evaluation, and different degree of self-absorption of the β -radiation in different tissues and different parts of the cell. Silver grains can also be produced in the emulsion by chemography (the chemical interaction of specimen and emulsion), by heat, by light, or by pressure. Even with a carefully standardized autoradiographic procedure, the intensity of the label can be modified by variation in the steps preparatory to autoradiography; for instance, the choice of developer, fixative, or the staining procedure. In order to minimize the effect of these different factors, all the experimental slides in this study were autoradiographed and developed together under precisely identical conditions.

Results and Discussion

Incorporation per cell of ^3H -uridine into RNA in hepatectomized mice (I)

Compared with normal liver, there is a lower uptake of ^3H -uridine into nuclear RNA in early regenerating liver. However, nuclear RNA labelling then rapidly increases to a maximum at about 36 h after hepatectomy and then declines. Also cytoplasmic RNA labelling, after an initial small decrease, reaches a maximum at about 12 h and then declines. Other investigators also reported, with biochemical methods, a decreased labelling of RNA or a drop in nuclear RNA content during early regeneration of rat (McArdle and Creaser, 1963; Tsanev and Markov, 1964) and mouse liver (Yngner et al., 1979) and also a decreased synthesis of low-molecular weight nuclear RNA in early regenerating rat liver (Stevely and White, 1970). They also reported that during later stages of regeneration in the rat and mouse liver, RNA incorporation and nuclear RNA content increased. The suppressed RNA labelling in early regenerating liver, as found in this study and also by others, is possibly concerned with the reorganization of cellular constituents in preparation for new cell growth, which takes place a few hours later. Evidence of a time-sequence for the activation of synthesis in regenerating liver, and of a delay in the synthesis of RNA until several hours after partial hepatectomy, is presented for rat liver (Glinos, 1958).

During the early period of regeneration, RNA species with a very rapid turnover are made (Mayfield and Bonner, 1972; McArdle, 1963). Within the 2 h long incorporation period used here, a great many such RNA species can therefore be assumed to have been broken down into nucleotides and therefore the delay in the increase in RNA labelling could also arise. Consistent with this opinion is the observation that

during this early period of regeneration, a heavily enhanced activity of nuclear RNase is seen (II), indicating an enhanced degradation of nuclear RNA at this period.

The 2 h-pulse period will probably result in most of the nuclear labelling residing in ribosomal precursor RNA (Bucher and Malt, 1971), as this RNA has a relatively slow turn over time (Hirsch and Hiatt, 1966). As the synthesis of ribosomal RNA (Drews and Brawerman, 1967; Loeb and Yeung, 1975) and the production of ribosomes (Morin-Coutu et al., 1977) are enhanced during rat liver regeneration, the great increase in total cell cytoplasmic RNA during mouse liver regeneration, as found in this study, should essentially reflect an increased production of ribosomes. This is to be expected as a corollary of increased synthesis of proteins during regeneration. Maximum cytoplasmic incorporation of RNA into regenerating rat liver was found at the same time as maximum protein-synthesising activity (Komar et al., 1969).

The amount of RNase-resistant labelling in nucleus and cytoplasm increased during the regenerative period. Most of it presumably represents DNA, as part of the labelling was removed by a following DNase-treatment. After RNase-treatment followed by DNase-treatment, less than 10 per cent of the labelling persisted. This labelling constitutes incorporation into RNase-resistant RNA or into cell compounds other than nucleic acids. Also in regenerating rat liver there are reports of incorporation into RNase-resistant RNA (Bonner and Huang, 1966; Bucher and Malt, 1971; Gotoh et al., 1971) or into cell compounds other than nucleic acids (Dahnke, 1975; Schneider and Greco, 1971).

Incorporation of ^3H -thymidine into DNA in hepatectomized mice (I)

After administration of ^3H -thymidine, DNA labelling was found

only over cell nuclei. In normal animals, 97 per cent of the cells were unlabelled and 3 per cent were weakly labelled. The labelled nuclei were equally distributed throughout the lobule. Immediately after partial hepatectomy, the percentage of weakly labelled cell nuclei increased. Heavily labelled nuclei appeared about 12 h after hepatectomy, but no sharp increase occurred until 24 h after the operation. Maximum incorporation occurred about 2 days after hepatectomy, as has also been reported from another autoradiographic study on regenerating mouse liver (Bade et al., 1966). The present study showed that labelled nuclei initially were concentrated near portal tracts (zone 1) and later during regeneration extending toward central veins (zone 3). Others also describe this zonal distribution of incorporated thymidine in regenerating rat liver lobule (Fabrikant, 1968; Grisham, 1962; Rabes and Brändle, 1969). Therefore, DNA synthesis and cell division seem to occur in sequences beginning in the outer zone and progressing in waves towards the central zone. Differences in metabolism of the three zones might help to explain these changes in the patterns of parenchymal cell proliferation and the utilization of available DNA precursor by proliferating cells (Brauer, 1963).

Comparison between ^3H -uridine and ^3H -thymidine incorporation in hepatectomized mice (I)

About 12 h after partial hepatectomy, there was maximum RNase-resistant uridine labelling in the nucleus and at 24 h in the cytoplasm. Faint labelling was also noted at this time in the nucleus after administration of ^3H -thymidine. In many different kinds of rapidly proliferating cells, to which regenerating liver cells belong, a special

kind of DNA with a rapid turn over is synthesized (Pelc, 1968). It was suggested that this metabolic DNA serves as a template for messenger-RNA production in the cytoplasm (Bell, 1969; 1971). The weakly labelled DNA that, in the present study, appears in the regenerating mouse liver before the S-phase (which begins at about 24 h and is characterized by heavily labelled nuclei) possibly conforms to this criteria.

Interestingly, van Tuyle and Kalf (1972) observed in normal rat liver the presence of a RNA-DNA complex in mitochondrial membranes. It was found that the percentage of labelled DNA in this complex was 3-4 times higher in regenerating liver compared with normal liver. Possibly, part of the RNase-resistant labelling found in this study could therefore depend on incorporation into this mitochondrial complex.

The amount of ^3H -cytidine, ^3H -uridine, and ^3H -orotic acid incorporated into normal and 24 h regenerating liver RNA, measured per unit area (III)

Total incorporation into RNA 20 and 300 m. after injection of ^3H -cytidine was about the same in normal and 24 h regenerating mouse liver. In both cases the cytoplasmic incorporation was only about 1 per cent compared with the nuclear. However, 20 m. after injection of ^3H -cytidine, the cytoplasmic RNA-labelling in regenerating liver was in fact twice that of normal liver. This increased fraction may represent labelled cytidine incorporated into RNA through the pathway via uracil nucleotides, as the activity of cytidine deaminase increases in mouse liver after partial hepatectomy (Rothman et al., 1971).

When ^3H -uridine, instead, was used as RNA-precursor, nuclear and cytoplasmic RNA labelling was lower in the liver of the regenerating animal than in controls. This decreased incorporation of labelled uridine into regenerating liver RNA is probably explained by increased

pools of uridine and UMP (uridine-mono-phosphate) in regenerating mouse liver (Yngner et al., 1979), thereby diluting the administered labelled uridine. No differences between normal and regenerating liver concerning incorporation into RNA were found after administration of ^3H -orotic acid. These autoradiographic results largely support the biochemical findings on the incorporation of these precursors into RNA in normal and regenerating mouse liver.

The cytoplasmic RNA labelling, calculated in per cent of total RNA labelling per cell, was much greater 20 m. after injection of ^3H -uridine than after injection of ^3H -cytidine or ^3H -orotic acid. It can also be seen from data by Schultze and Maurer, (1963) that, soon after the injection of RNA-precursors, the per cent cytoplasmic RNA labelling of different mouse tissues was higher after the injection of ^3H -uridine than of ^3H -cytidine. It seems likely that the differences between the precursors cannot be attributed solely to differences in the rate of uptake and metabolism in the cells. The considerably higher cytoplasmic RNA labelling obtained 20 m. after the ^3H -uridine injection might be interpreted as indicating that this precursor is incorporated preferentially into RNA species (probably messenger-RNA) that are transported from nucleus to cytoplasm more rapidly than RNA labelled with cytidine and orotic acid. Such a condition would probably require separate uracil nucleotide pools, supplied to a different extent with precursors via the de novo and the salvage pathways and providing precursors for different RNA species. The existence of separate nucleotide pools utilized for synthesis of messenger-RNA and ribosomal-RNA has been indicated in HeLa cells (Wieggers et al., 1976).

The cellular distribution of newly synthesized RNA is similar in normal and regenerating mouse liver and the same distribution was also

found in regenerating rat liver by Stöcker (1968). This could indicate that the quantitative transport of the newly synthesized RNA from nucleus to cytoplasm is not altered after partial hepatectomy.

The activity of RNase during mouse liver regeneration (II)

The nuclear RNase-activity in hepatectomized animals was highly enhanced during the early period of regeneration. Also in the rat, it has been found that, in the early period after partial hepatectomy, there was an increased activity of RNA-degrading enzymes present in nucleoli (Schmid and Sekeris, 1975). This early activation of nuclear RNase could be related to the degradation of the old messenger RNAs during the regressive phase of regeneration. When an increased need for protein synthesis is imposed on the cell that requires the synthesis of new types and the breakdown of old types of proteins, these conditions would call for the breakdown of no-longer needed messenger-RNAs, and the degradation of these RNAs would require the action of nuclear RNases.

There was no significant change in the cytoplasmic RNase-activity in regenerating, compared with normal mouse liver. It is therefore suggested that the activity of this cytoplasmic enzyme does not play any significant role for the regenerative process in the mouse liver. In present investigation, the activity of both nuclear and cytoplasmic RNase varied in accordance with the used nucleus-isolation procedure. Therefore, the activity of RNases seems to be greatly influenced by the occurrence and concentration of cations or other factors in the medium. As was to be expected, the results obtained by various authors on the RNase-activity in regenerating rat liver are not in agreement. The discrepancies can therefore be explained by the use of different methods of assay of the RNase-activity.

Comparison between the incorporation of ^3H -uridine into regenerating liver RNA when measured per cell (I) and per unit area (III)

During the livers regenerative process after partial hepatectomy the liver cell enlarges in size (Bucher and Malt, 1971). After administration of ^3H -uridine to normal and 24 h regenerating mouse liver, its incorporation into regenerating liver nuclear and cytoplasmic RNA was increased when measured per cell (I). However, when measured per unit area (III), incorporation of this precursor into RNA was not increased. In the latter study, the incorporation of ^3H -uridine into normal and 24 h regenerating liver nuclear and cytoplasmic RNA was also measured per cell (not shown). The pronounced increased incorporation of ^3H -uridine into 24 h regenerating nuclear and cytoplasmic liver RNA, as found in (I), was not repeated in (III). However, the two studies were not exactly comparable, and this perhaps explains the differences. The discrepancy could be due to the use of experimental animals oppositely sexed in the two studies. It is well known (Feigelson and Feigelson, 1964; Drews and Brawerman, 1967; Yu and Feigelson, 1970), that glucocorticoids augment the incorporation of radioactivity from labelled precursors into hepatic RNA. This could possibly reflect an increased rate of RNA synthesis, but also an influence of the hormone in altering the metabolic activity of the precursor pool. Therefore, in the present study, difference in steroid hormone action between the sexes might explain the different results on the incorporation into RNA.

Comparison between regenerating rat and mouse liver on the incorporation into RNA

Rat liver shows an early increase in the incorporation of labelled precursors into RNA after partial hepatectomy, measured biochemically

(Bresnick, 1971; Bucher and Malt, 1971) and autoradiographically; per unit area (Rabes and Brändle, 1969; Stöcker, 1968). However, as demonstrated in paper III, which conforms to biochemical results at this laboratory, the regenerating mouse liver does not show this substantially increased incorporation of labelled precursors into RNA. (The actually increased observation in paper I has already been discussed). There is also a different utilization of the precursors for incorporation into RNA in normal rat (Dahnke, 1975) and mouse liver (Yngner, 1979). The difference between rat and mouse liver can depend on a different enzymatic equipment for metabolism of the administered pyrimidine precursors (Reichard and Sköld, 1958). It is also possible that the synthesis of RNA in mouse liver, contrary to rat liver, is not significantly increased after partial hepatectomy. Instead, the demands imposed by regeneration to increase the amount of RNA might be met by only moderate new synthesis combined with a less pronounced catabolism of the old RNA. In this connection it is interesting to observe that during compensatory renal hypertrophy, there was an increased incorporation of labelled precursors into rat kidney RNA (Bucher and Malt, 1971; Halliburton, 1969), but not into mouse kidney RNA (Hill et al., 1974).

Summary and Conclusions

Rats are chosen for most studies on liver regeneration. This study chose the mouse in order to find differences and similarities between the two species. As synthesis and turn over of RNAs seem to be of great importance for the regenerative process in the liver after partial hepatectomy, the present investigation studied the synthesis of RNA (and to some degree of DNA) and the activity of nuclear and cytoplasmic RNase in mouse liver after partial hepatectomy.

The autoradiographic method was to study the incorporation of ^3H -uridine into total cell nuclear and cytoplasmic RNA of liver parenchymal cells after partial hepatectomy. After a decreased uptake of uridine into nuclear and cytoplasmic RNA during the first hours, incorporation increased to a maximum and then declined. After RNase-digestion of the liver sections, a small incorporation was left both in nucleus and cytoplasm. This was presumably RNase-resistant RNA, but it cannot be excluded that it was DNA or compounds other than nucleic acids.

There was a small amount of incorporation of ^3H -thymidine into DNA immediately after partial hepatectomy, but a marked uptake of this precursor was seen 24 h after the operation. This time is generally accepted as the beginning of the S-phase in regenerating mouse liver. The faint labelling of thymidine before the S-phase was correlated with incorporation of RNase-resistant uridine at the same time. This incorporation could probably reside in metabolic or mitochondrial DNA.

Autoradiographic and biochemical methods were used to study the incorporation of ^3H -labelled uridine, cytidine, and orotic acid into liver RNA 24 h after partial hepatectomy. Nuclear and cytoplasmic RNA labelling, measured autoradiographically per unit area, was decreased in regenerating liver after injection of ^3H -uridine. This decreased incorporation can be related to increased pools of uridine in regenerating mouse liver, thereby diluting the administered labelled uridine. Nuclear RNA labelling was about the same in normal and regenerating liver after injection of ^3H -cytidine. However, cytoplasmic RNA labelling, which was only c. 1 per cent of the nuclear, was twice as high in regenerating as in normal liver. This is explained as being due to the incorporation of an increased fraction of labelled cytidine into RNA via uracil nucleotides. No difference between normal and regenerating liver was found on the incorporation into RNA after injection of ^3H -orotic acid.

These autoradiographic results support the biochemical results that none of the precursors has an increased incorporation into cellular RNA 24 h after partial hepatectomy.

Cytoplasmic RNA labelling calculated in per cent of total RNA labelling per cell, was much greater 20 m. after injection of ^3H -uridine than after injection of ^3H -cytidine or ^3H -orotic acid. This was interpreted as indicating that uridine is preferentially incorporated into RNA species (probably m-RNA) that are transported from nucleus to cytoplasm more rapidly than RNA labelled with cytidine and orotic acid.

The decreased incorporation of uridine into RNA that was found during early regeneration was suggested to be correlated to an

enhanced activity of nuclear RNase at this time. This early activation of nuclear RNase could be related to the degradation of the old m-RNAs during the regressive phase of regeneration.

There was no change in cytoplasmic RNase-activity in regenerating mouse liver. It was therefore suggested that the activity of this cytoplasmic enzyme does not play any role for the regenerative process in mouse liver.

Rat liver shows an early increase in the incorporation of labelled precursors into RNA, measured per unit amount of tissue, after partial hepatectomy. However, the present study shows that there is no increased incorporation into RNA in regenerating mouse liver. It was therefore suggested that the demands imposed by regeneration on the increased amount of RNA in mouse liver does not necessitate very large changes in total RNA production. Instead, change in stability leading to less pronounced turn over of RNA is perhaps sufficient.

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